

data acquisition and processing including 3D reconstruction, which is not dependent on symmetry. Prior art solutions have not yet overcome the noise problem from intermediate (below 15 Å) to high (below 7 Å) resolutions without the need for symmetry to manage the calculations.

DESCRIPTION OF RELATED ART

A method of multi-scale reconstruction of the image of the structure of a body at an increased speed using iterative algorithms is disclosed in the U.S. Pat. No. 5,241,471. The algorithms are constructed such that the image of the object to be reconstructed is done on a structure, for instance a grid, having a finer and finer finesse from iteration to iteration. In this way the calculations could be done very fast. Thus, it will not work with fine details from the start and do not count on components having a resolution higher than the resolution of the structure (grid) actually in the iteration. No density distribution for individual grid points is done. The description of the sample on the grid is thus not changed from iteration to iteration, only its resolution.

An approach for providing a solution of this problem is to use maximum entropy. This is described by G. J. Ericksom & C. R. Smith, *Maximum-Entropy and Bayesian methods in science and engineering*, Volume 1: Foundations 1-314 (Kluwer Academic Publishers, Dordrecht, The Netherlands (1988)), by C. R. Smith & W. T. Grandy Jr, *Maximum-Entropy and Bayesian methods in inverse problems*, p 1-492 (D. Reidel Publishing Company, Dordrecht, Netherlands (1985)), and by B. Buck & V. A. Macaulay, 220 (Oxford University Press, New York (1991)). A maximum entropy 3D reconstruction has the property of being maximally noncommittal with regard to missing information (E. T. Jaynes, *Physical Review* 106, p 620-630 (1957)), and thus the maximum entropy method could serve as a powerful method to remove some of the detrimental effects caused by missing projections in ET reconstructions.

A method to perform a 3D reconstruction of an object with high resolution is described by Ali Mohammad-Djafari et al, "Maximum Entropy Image Reconstructions in X-Ray and Diffraction Tomography", *IEEE Transactions on Medical Imaging* Vol. 7 (December 1988) No 4, New York USA, PG. 345-354.

The argument and the applications in this reference refer only to a reconstruction from 1D to 2D, because of the idea that in medical imaging one reconstructs each circular "slice" independently from the others. Thus only 2D slice reconstructions are performed and the 3D is provided by adding the slices together. Thus the slices are treated as independent from each other. This method of treating the slices as independent gives unpredictable result when deconvoluting the real 3D point-spread function and possible line broadening. The result provided with the method described in this article can therefore not be quantitatively correct. When using the Poisson statistical properties of the image this is then not correct since it emanates from the a large part of the specimen, since the treatment of it is as if it were a local phenomenon restricted to a 1D line.

The implementations of the maximum entropy principles in ET 3D reconstructions have aimed at maximising the entropy while under the constraint that the reduced chi-squared statistic be equal to 1.0 as suggested by M. Barth, R. K. Bryan, R. Hegerl & W. Baumeister, *Scanning Microsc. Suppl.* 2, p 277-284 (1988) and by M. C. Lawrence, M. A. Jaffer & B. T. Sewell, *Ultramicroscopy* 31, p 285-301 (1989).

The usefulness of assigning low weight to improbable situations, so that the iterative solution of the problem

becomes that of maximising the entropy relative to the non-informative prejudice, i.e. an estimated 3D prejudice distribution of the density, under the constraints of making the reduced chi-squared statistic equal to +1, has been shown by Gerard Bricogne in the article 'Maximum entropy and the foundations of direct methods' in *Acta Crystallographica A* 40, pp410-445 (1984), which discloses an algorithm used for constrained entropy maximisation for crystallographic phase refinement against reciprocal space data. However, the mathematics shown by G. Bricogne can not be applied on the tomographic problem. His method is a development of so called direct methods within crystallography which give a generalized solution of a statistical problem and does not deal with imaging problems. A diffraction pattern is provided which is the diffraction data from crystallographic examinations giving focal planes, and thus no image is provided. When several generated focal planes are collected, for instance on a photographic film or detector, then the phase information of the different arriving waves is lacking. The crystallographic problem is aimed at finding the phase information so that an image can then be calculated.

Thus the problem in crystallography is different to that of 3D imaging of macromolecular complexes lacking symmetry, which is solved by the invention. However, the tools that have been developed to be used for providing a high fidelity reconstruction in real space could be the knowledge that it is important to have a constrained maximum entropy formalism and to use Taylor expansion of the chi-squared statistic and the entropy as quadratic models.

This kind of direct method described by Gerard Bricogne is directed to solve the phase problem in crystallography and is therefore not applicable in 3D imaging of macromolecules lacking symmetry. Therefore, the whole concept had to be totally redesigned in order to perform for the real space imaging problem. The algorithm used for constrained entropy maximisation was thus first devised for crystallographic phase refinement against reciprocal space data. It was adapted to the tomographic situation by replacing the Fourier transformation which relates molecular model to diffraction data in the crystallographic setting by line projections in real space. However, it could not handle projections at any angle. The problem was to provide a method which was independent of specific data sampling strategies like single-axis tilt series, conical tilt series, random tilt series, or tilts from symmetric samples where the projection angles could be calculated later (as is the case for the adenovirus). This technical problem has now been solved according to the invention.

OBJECTS OF THE INVENTION

Is is an object of the invention to provide high fidelity reconstruction of an observed sample, for instance by 3D imaging, practically independent of the size of the object.

It is an object of the invention to overcome the technical problems mentioned above, concerned with 3D imaging of single particle macromolecular complexes at high resolution, with a high signal to noise ratio.

It is a further object of the invention to provide a method and an apparatus which is independent of the object's symmetry and which also offers the possibility of removing detrimental effects during the reconstruction of missing data.

It is also an object of the present invention to provide a 3D image reconstruction of single particle macromolecular objects lacking symmetry, with a high enough resolution to make it possible to reconstruct a 3D image of macromolecular objects lacking symmetry, using a series of 2D projections recorded with an electron microscope or a similar means.